REMARKS

Claims 1, 17, 24 and 26-40 are now pending. Upon entry of this amendment, claims 1, 24, and 28-39 will be amended. These amendments are discussed below with respect to particular objections or rejections. In addition, claim 40 will be cancelled. No new matter has been added and no new issues have been raised. Entry of this amendment is therefore respectfully requested.

Applicants gratefully acknowledge that the Examiner has viewed the claims as properly enabled.

Objections to the Specification

The application has been amended to delete reference to U.S. provisional application Serial Nos. 60/161,141 and 60/206,082 in the cross-reference to related applications; instead, reference to these applications, which do describe subject matter related to the present invention, has been made at page 92 of the specification. Applicants therefore request that this objection to the specification be withdrawn. No new matter has been added as these applications were incorporated by reference in the originally filed application.

The Examiner noted that although Applicants amended the specification to include new Figures 2-9, Applicant did not include the filing of such Figures. Applicants forwarded these Figures to the Office on May 14, 2002. The Office date-stamped the post card on May 14, 2002, demonstrating that the Office received the Figures. A copy of Applicant's submission relating to the Figures on May 14, 2002, is attached herewith (Exhibit A). The application has now been amended specifically to include these Figures (Figures 2-9) after Figure 1. In addition, all references to graphs that have been moved from the specifications to a Figure have been removed or corrected to indicate that the graph is no longer located in the specification but rather is in a Figure. The Applicants appreciate the Examiner's careful review of the specification and apologize for the oversight in not correcting such references at the time the new Figures were

submitted. Also, a Request for Correction of Drawings has been submitted on same date as herewith to amend Figure 4 from a color graph to one that contains distinguishing lines in black and white, and the description of the Figure has been amended accordingly in the specification. As Applicant has corrected such references and Figure 4, withdrawal of the objections to the specification is requested.

Applicants have deleted the phrase in Claims 30, 34, and 38 "from a gene of an *E. coli* phage" and gratefully acknowledge the Examiner's recognition that a promoter of an "*E. coli* gene" would include an *E. coli* phage promoter. For the record, Applicants direct the Examiner's attention to page 28, lines 18-22 of the present specification for support of "a gene from *E. coli* phage." Because of the Examiner's statement, however, the issue is moot, and the phrase objected to by the Examiner has been deleted. Applicants therefore request that the objection to the claims under 35 U.S.C. § 132 and the rejections under 35 U.S.C. §112, first and second paragraphs, be withdrawn.

Claim Objections

Claims 30, 34, and 38 to which the Office objects have been amended as discussed above, to delete the phrase to which the Examiner objects (i.e. "from a gene of an *E. coli* phage") such that these objections are moot. In addition, Claims 30, 34, and 38 to which the Office objects due to typographical error have been amended to delete the period after "*E. coli*". The Applicants therefore request that the objections to these claims be withdrawn.

Claim Rejections - 35 U.S.C. § 112

Indefiniteness:

Claims 1, 17, and 26-35 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter with regard to the phrase "for biosynthesis of a polyketide produced by a modular polyketide

synthase (PKS) produced by a PKS gene or genes in said host cell" as unclear. The Applicants have added clarifying language to the claims such that the PKS gene or genes are defined as "said PKS gene or genes contained in a vector that replicates extrachromosomally or is integrated into chromosomal DNA." Support for this amendment is found, for example, on page 27, lines 20-25 of the present specification. The Applicants therefore respectfully request withdrawal of this rejection. Applicants have also changed the language from " produced by" to "expressed from" to clarify the claim. Support for this amendment is found, for example, on page 28, lines 15-18 of the present specification.

Claims 24 and 36-39 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter with regard to the phrase "wherein said genes produce enzymes capable of making S-methylmalonyl CoA, and a modular polyketide synthase (PKS) gene" as unclear. Claim 24, upon which claims 36-39 depend, has been amended to make clear that the mutase and epimerase genes produce the enzymes capable of making S-mmCoA, and to delineate those genes from the gene or genes that express the PKS. The Applicants therefore respectfully request withdrawal of this rejection.

Claims 28, 29, 32, 33, 36, and 37 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter in referring to a mm-CoA mutase gene as singular when the specification shows that two genes, the mutA and mutB genes are required for this activity. Applicants have amended these claims to refer to two mm-CoA mutase genes, *mutA* and *mutB*, and made reference to mm-CoA mutase genes in the plural to denote more than one gene. The claims are amended to state the mutA and mutB genes come from *Propionibacterium shermanii* or *Streptomyces cinnamonensis* Thus, withdrawal of this rejection is requested.

Claims 28, 29, 32, 33, 36, and 37 are further rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter with regard to the lack of clarity of the phrase "**the** *Propionibacterium*

shermanii methylmalonyl CoA mutase gene" and "**the** *Streptomyces cinnamonensis* methylmalonyl CoA mutase gene" as allegedly unclear. These claims have been amended as described in the preceding paragraph to delete "the" and thus are respectfully submitted to overcome this rejection. Withdrawal of this rejection is therefore respectfully requested.

Claims 30, 34, and 38 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter with regard to the phrase "from a gene of an *E. coli* phage" as being unclear. As noted above, support for *E. coli* phage promoters can be found on page 28 of the specification, but because the Examiner acknowledges that the phrase "an *E. coli* gene" in claims 30, 34 and 38 includes a "gene from an *E. coli* phage," the Applicants have removed reference to phage promoters, and respectfully request that this rejection be withdrawn.

Claims 31, 35, and 39 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter with regard to the phrase "PKS gene is a gene that encodes a 6-dEB synthase protein" as unclear, because 6-dEB synthase is produced by three genes. The Applicants have clarified the instant claims such the phrase "said PKS is 6-deoxyerythronolide B synthase" no longer appears to refer to the synthase as the product of a single gene. The Examiner will appreciate that claim 1 and the other independent claims all now refer to "PKS gene or genes," and thus is consistent with a reference to a PKS that requires that more than one PKS gene be present in the host cell. Thus, the amended claim language is definite, and the Applicants respectfully request that this rejection be withdrawn.

Written Description:

Claims 1, 17, 24, and 26-39 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which allegedly was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention with regard to "any" mutase gene

from *P. shermanii* or S. *cinnamonensis* and "any" epimerase gene from *P. shermanii*. The Applicants respectfully traverse this rejection, pointing out that the specification adequately describes these genes with particularity beginning at page 15. In addition, Example 1, beginning on page 51 of the present specification, includes cloning and expression vectors and methods and activity assays, for both *P. shermanii* methylmalonyl CoA mutase and epimerase, and further, Example 1 describes the exact sequence of the *P. shermanii* epimerase and the coding sequence therefor. Thus, there is sufficient support in the specification such that a person of ordinary skill in the art would understand that the inventor was in possession of the invention set forth in the claims.

Moreover, it is incumbent upon the Examiner to explain why she doubts the truth or accuracy of the specification and provide a reasonable basis for such doubt. *In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (CCPA 1971). The Examiner's basis for the instant rejection appears to be mere conjecture: there may be mutase or epimerase genes in *P. shermanii* or S. *cinnamonensis*, in addition to those described in the specification, and one of ordinary skill in the art would be unable to recognize them or utilize them despite the teachings of the instant application. The Applicants respectfully submit that the Examiner has not established a reasonable basis for any of these doubts. The Applicants respectfully believe that this rejection has therefore unfairly shifted the burden of proof to them. As such, this rejection fails to rise to the level of a *prima facie* case and should be withdrawn.

The present facts are analogous to the facts in *Regents of the Univ. of Ca. v. Eli Lilly*, 119 F3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997), wherein rat cDNA encoding insulin provided sufficient written description. Similarly, the Applicants have provided literature references showing a skilled person would understand that the inventors had possession of the mutase genes in *P. shermanii* and S. *cinnamonensis* and have provided the coding sequence for the epimerase gene in *P. shermanii*. No question was raised in *Eli Lilly* that rat contained another insulin gene thus rendering the "rat cDNA" lacking in proper written description. Likewise, it is respectfully submitted that no question should be raised here. If, however, there is personal knowledge of

facts upon which the Examiner relies, she is kindly requested to support such facts in accordance with MPEP §2144.03. Otherwise, the Applicants respectfully submit that the claims properly comply with the written description requirements and request that the Examiner withdraw this rejection under 35 U.S.C. § 112, first paragraph.

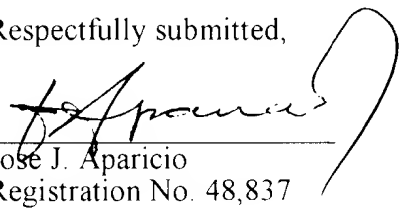
The Applicants also respectfully request the Examiner to consider that, as the claims now refer to genes that are either published in scientific journals or disclosed in the specification, there can be no doubt that the specification contains appropriate written description with respect to such genes. If there are additional mutase or epimerase genes in the organisms recited in the claims, then there appears to be no reason why one of ordinary skill, given the teachings of the instant specification, could not employ them as contemplated by the specification to make and use the claimed invention. To the extent that the Examiner is rejecting the instant claims because the specification lacks teaching of how to find genes in those organisms that the Applicants do not necessarily believe exist, the Applicants respectfully submit that the claimed invention is not a method to find genes. Because one or ordinary skill in the art has access to the genes recited in the instant claims, and because there is no basis for asserting that additional genes exist that would not be obtainable by one of ordinary skill, the rejection under 35 U.S.C. § 112, first paragraph, should be withdrawn.

Claims 30, 34, and 38 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention, with regard to the phrase "from a gene of an *E. coli* phage". Applicants respectfully point out that a patent application need not teach and preferably omits what is well known in the art, and while experimentation may be complex it is not necessarily undue. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). It is improper to conclude that a disclosure is not enabling based on an analysis of only one of the 8 *In re Wands* factors while ignoring one or more of the factors. *Id.* at 740. In order to make a proper rejection, the examiner has the initial burden to establish a reasonable basis to question the enablement

provided for the claimed invention. For the reasons noted above, it is respectfully requested that this rejection be withdrawn.

In conclusion, it is respectfully submitted that the claims have been amended such that the claims are in condition for allowance. Such action is respectfully requested.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please amend the paragraph on page 1 commencing at line 5, as follows:

This patent application claims priority to U.S. patent application Serial No. 60/161,703, filed 27 Oct. 1999, [and is related to Serial Nos. 60/161,414, filed 215 Oct. 1999, and 60/206,082, filed 18 May 2000, each of]which is incorporated herein by reference.

Please amend the paragraph on page 56, commencing at line 9, as follows:

[The graph below] Figure No. 3 shows the comparison of *in vivo* acyl-CoA levels in BL21(DE3) *pan*Dstrains with and without mm-CoA mutase. For each CoA, the ratio of the amount in the strain containing the mutase to the amount in the control strain was determined. Interestingly, malonyl-CoA was increased about 25-fold and succinyl-CoA about 3-fold. Acetyl-CoA and CoA were increased just slightly, and propionyl-CoA was not detected in either case.

Please amend the paragraph on page 61, commencing at line 7, as follows:

[The graph below]Figure No. 4 shows the comparison of *in vivo* acyl-CoA levels with and without the mutase and with and without hydroxocobalamin. In the cells over-expressing the mutase and grown with hydroxocobalamin, methylmalonyl-CoA comprised 13% of the overall CoA pool, whereas in the other cells no methylmalonyl-CoA was detectable. The background level of counts is about 0.25% of the overall number of counts in the CoAs, suggesting that any methymalonyl-CoA present in *E. coli* strains not over-expressing the mutase would comprise at most 0.25% of the overall CoA pool, or 2% of the amount of methylmalonyl-CoA observed in the strain over-expressing the mutase. The composition of the CoA pool observed for the *E. coli panD* strain is consistent with that observed previously for *E. coli panD* mutants grown on glucose.

Please amend the paragraph on page 62, commencing at line 2, as follows:

[The graph above]Figure 4 shows the results of CoA analysis of *E. coli* over-expressing methylmalonyl-CoA mutase. The levels of ^{3}H detected in fractions collected from HPLC of cell-free extracts from ^{3}H ß-alanine-fed *E. coli* harboring either the pET control vector grown without hydroxocobalamin (solid line[black trace]), pET grown with hydroxocobalamin (short dash[blue trace]), pET over-expressing the mutase and grown without hydroxocobalamin (long dash[green trace]), or pET over-expressing the mutase and grown with hydroxocobalamin (medium dash[red trace]) are shown.

Please amend the paragraph on page 81, line 17 through page 82, line 3, as follows:

As described in Example 1, the translationally coupled genes, *mutA* and *mutB*, encoding the ß - and α-subunits of methylmalonyl-CoA mutase from *Propionibacterium shermanii*, were amplified by PCR and inserted into an *E. coli* expression vector containing a T-7 promoter. The naturally occurring GTG start codon for *mut*B was changed to ATG to facilitate expression [5]. Heterologous expression of the mutase genes in media containing [^{3}H] ß-alanine and the adenosylcobalamin (coenzyme B_{12}) precursor, hydroxocobalamin, yielded active methylmalonyl-CoA mutase. HPLC analysis of extracts from *E. coli* BL21(DE3)/*pan*D harboring the mutase genes indicated production of methylmalonyl-CoA, which comprised 13% of the intracellular CoA pool (shown in Figure 6 [below]). This work demonstrates that one can introduce a biosynthetic pathway for an important PKS substrate into a heterologous host, and that one can measure the intracellular concentration of acyl-CoAs. In accordance with the present invention, the methylmalonyl-CoA mutase gene (*sbm*) from *E. coli*, which has codon usage closer to yeast and encodes a single polypeptide [16], can also be employed.

Please amend the paragraph on page 83, commencing at line 1, as follows:

Propionyl-CoA is not detected in *E. coli* SJ16 cells grown in the presence of [^{3}H] ß-alanine with or without the addition of propionate in the growth media. When *E. coli* SJ16 cells

were transformed with a pACYC-derived plasmid containing the *Salmonella typhimurium* propionyl-CoA ligase gene (*prpE*) under the control of the *lac* promoter, a small amount of propionyl-CoA was observed (~0.2% of total CoA pool) in cell extracts. When 5 mM sodium propionate was included in the culture medium, about 14-fold more propionyl-CoA was produced (~ 3% of the total CoA pool). [These results are shown graphically below.]

Please amend the paragraph on page 85, commencing at line 5, as follows:

Genes involved in the production of substrates (e.g. methylmalonyl-CoA and/or propionyl-CoA), and the *sfp* gene can preferably be stably integrated into the yeast chromosome in appropriate copy number to produce adequate levels of desired acyl-CoAs and post translational PKS modifications. Genes can first be introduced into the intermediate Bluescript cloning vector as described. Then, the fragment containing the promoter-gene-terminator cassette can be transferred as a L1-L3 fragment to a yeast "delta integration" vector [18] [19] that allows chromosomal integration of the cassettes into one or more of the ca. 425 delta sequences dispersed throughout the yeast chromosome (see [the schematic below]Figure 9). These vectors have cloning sites compatible with those in the L1-L3 linkers to permit direct transfer of promoter-gene-terminator cassettes as L1-L3 fragments. They also contain the excisable Ura3 selection marker flanked by two bacterial *hisG* repeats ("URA Blaster"), enabling insertion of multiple identical or different genes into the yeast chromosome by repetitive integration's. After selection for gene integration on media lacking uracil, the Ura3 gene fragment is removed by selecting for marker loss via excisional recombination by positive selection with 5-fluoroorotic acid (FOA), which renders the Ura3 gene toxic to yeast. This enables the introduction of stable pathways needed for acyl-CoA precursors and Sfp into yeast, while conserving the Ura marker to allow its subsequent use in plasmids containing other genes.

Please amend the paragraph on page 85, line 25, through page 86, line 7, as follows:

The single-gene mutase, Sbm (Sleeping beauty mutase), from *E. coli* [16], can be cloned as follows. Primers designed based on the DNA sequence were used to PCR amplify the *sbm* gene from *E. coli* genomic DNA as a *Nde*I-L2 fragment. The general strategy for cloning the genes into yeast expression vectors follows that of Kealey *et al.* [3] (see [the schematic below]Figure 9). One can first clone the genes as *Nde*I-L2 fragments into the intermediate Bluescript cloning vector. The promoter-gene-terminator cassette can then be excised as an L1-L3 fragment, transferred to the yeast integrating vector, restricted with L1/L3, and introduced into the yeast chromosome as described above. As an alternative to Sbm, one can use the two-gene mutase from *P. shermanii*; the translationally coupled genes have each been amplified by PCR as *Nde*I-L2 fragments and can be integrated into yeast as described above.

Please amend the paragraph on page 86, commencing at line 14, as follows:

The *pccB* and *accA1* genes involved in the propionyl-CoA carboxylation pathway in *S. coelicolor* can be amplified by PCR from genomic DNA. As shown in [the schematic below]Figure 9, the genes can be cloned into the intermediate Bluescript vector between *Nde* I and L2, then transferred to the yeast integrating vector via L1/L3. One can express the *S. coelicolor* genes shown to be effective in *E. coli*; should codon usage be suboptimal, one can employ the *B. subtilis* orthologs (discussed above).

In the Claims:

Please amend the following claims:

1. (Twice Amended) A recombinant *E. coli* host cell comprising one or more expression vectors that comprise

[a] methylmalonyl CoA mutase genes mutA and mutB [selected] from either [the group consisting of a] *Propionibacterium shermanii* or [methylmalonyl CoA mutase gene and a] *Streptomyces cinnamonensis* [methylmalonyl CoA mutase gene], and

a *Propionibacterium shermanii* epimerase gene,

wherein said genes produce enzymes capable of making S-methylmalonyl CoA required for biosynthesis of a polyketide produced by a modular polyketide synthase (PKS) expressed from a PKS gene or genes in said host cell,

said PKS gene or genes contained in a vector that replicates extrachromosomally or is integrated into chromosomal DNA,

wherein said host cell, in the absence of said expression vectors, is unable to make said polyketide due to lacking all or a part of a biosynthetic pathway required to produce S-methylmalonyl CoA.

24. (Twice Amended) An *E. coli* host cell that expresses

[a] methylmalonyl CoA mutase genes mutA and mutB [selected] from either [the group consisting of a] *Propionibacterium shermanii* [methylmalonyl CoA mutase gene and a] or *Streptomyces cinnamonensis* [methylmalonyl CoA mutase gene], and

a *Propionibacterium shermanii* epimerase gene,

wherein said mutase and epimerase genes produce enzymes capable of making S-methylmalonyl CoA, and

said host cell further expresses a modular polyketide synthase (PKS) gene[.] or genes,

said PKS gene or genes contained in a vector that replicates extrachromosomally or is integrated into chromosomal DNA.

28. (Amended) The host cell of Claim 1, wherein said methylmalonyl CoA mutase genes are [is the] *Propionibacterium shermanii* methylmalonyl CoA mutase genes mutA and mutB.

29. (Amended) The host cell of Claim 1, wherein said methylmalonyl CoA mutase genes are [is the] *Streptomyces cinnamonensis* methylmalonyl CoA mutase genes mutA and mutB.

30. (Amended) The host cell of Claim 1, wherein one or more of said genes is under control of a promoter from an *E. coli* gene[or from a gene of an *E. coli* phage].

31. (Amended) The host cell of Claim 1, wherein said PKS is[a gene that encodes a] 6-deoxyerythronolide B [(6-dEB)] synthase[protein].

32. (Amended) The host cell of Claim 17, wherein said methylmalonyl CoA mutase genes are [is the] *Propionibacterium shermanii* methylmalonyl CoA mutase genes mutA and mutB.

33. (Amended) The host cell of Claim 17, wherein said methylmalonyl CoA mutase genes are [is the] *Streptomyces cinnamonensis* methylmalonyl CoA mutase genes mutA and mutB.

34. (Amended) The host cell of Claim 17, wherein one or more of said genes is under control of a promoter from an *E. coli* gene[or from a gene of an *E. coli.* phage].

35. (Amended) The host cell of Claim 17, wherein said PKS is[a gene that encodes a 6-dEB] 6-deoxyerythronolide B synthase[protein].

36. (Amended) The host cell of Claim 24, wherein said methylmalonyl CoA mutase genes are [is the] *Propionibacterium shermanii* methylmalonyl CoA mutase genes mutA and mutB.

37. (Amended) The host cell of Claim 24, wherein said methylmalonyl CoA mutase genes are [is the] *Streptomyces cinnamonensis* methylmalonyl CoA mutase genes mutA and mutB.

38. (Amended) The host cell of Claim 24, wherein one or more of said genes is under control of a promoter from an *E. coli* gene[or from a gene of an *E. coli.* phage].

39. (Amended) The host cell of Claim 24, wherein said PKS [gene] is [a gene that encodes a 6-dEB] 6-deoxyerythronolide B synthase[protein].